FINAL

DATA EVALUATION REPORT

OXINE COPPER

Study Type: Developmental Toxicity (Rat)

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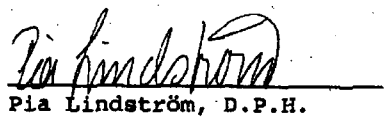
Principal Reviewer  Date 9/30/94
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[OXINE COPPER] Developmental Study (83-3)

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DATA EVALUATION RECORD

STUDY TYPE: Developmental Study - Rat (83-3)

TOX CHEM. NO.: 253

P.C. CODE: 024002

MRID NUMBER: 429868-03

TEST MATERIAL: Ro 17-0099/000

SYNONYMS: Copper 8-Quinolinolate; Oxine Copper; Oxine-Cu; Oxine cuivre; Oxyquinolinoleate de cuivre; Bioquin; Cunilate 2472; Dokirin; Fruitdo; bis (8-quinolinolate) copper; oxine copper

STUDY NUMBER: B-154'980

SPONSOR: La Quinoleine et ses dérivés, S.A.

TESTING FACILITY: F. Hoffmann-La Roche Ltd., Basel, Switzerland

TITLE OF REPORT: Ro 17-0099/000 (Copper 8-Quinolinolate): Oral (Gavage) Embryo Toxicity Study in the Rat with the Fungicide Ro 17-0099/000. Segment II Study with Post Natal Evaluation

AUTHOR: C. Bacchus

REPORT ISSUED: January 13, 1992

EXECUTIVE SUMMARY: In a developmental toxicity study (including postnatal evaluations), 36 Fü-Albino rats per group received Ro 17-0099/000 by gavage on gestational days (GDs) 6-15 inclusive, at dose levels of 0, 50, 200, or 800 mg/kg/day. At least 20 dams per group were sacrificed on GD 20 and litters were delivered by cesarean section. The remaining dams were allowed to deliver naturally and rear their pups through lactational day (LD) 23. Aqueous carboxymethylcellulose (0.5%) served as the control substance and vehicle for the test article. The purity of the test substance was >98.5%. The study authors did not indicate if doses were adjusted for concentration of active ingredient.

Maternal toxicity was observed at 800 mg/kg/day as evidenced by increased clinical signs (piloerection, poor general condition, and encrustation of nose and mouth) and significantly decreased body weight on GD 16 (93% of control) and weight gain during the dosing period (48% of control). Based on these results, the **Maternal Toxicity NOEL was 200 mg/kg/day, and the Maternal Toxicity LOEL was 800 mg/kg/day.**

No developmental toxicity was observed. Consequently, the Developmental Toxicity NOEL was 800 mg/kg/day; the Developmental Toxicity LOEL was not determined.

Classification: Core Guideline Data. This study satisfies the requirements for a developmental study (83-3) in rats.

Special Review Criteria (40 CFR 154.7): None

MATERIALS and METHODS

A. MATERIALS

Test material:	Ro 17-0099/000
Description:	Not reported
Lot number:	8293/3
Purity:	>98.5%
Stability:	Two years at room temperature
CAS number:	Not reported
Vehicle:	0.5% Aqueous carboxymethylcellulose

Test Animals

Species:	Rat
Strain:	Fü-Albino
Age:	Not reported
Weight:	172-227 g on GD 0
Source:	BRL, Biological Research Laboratories Ltd., Füllinsdorf, Switzerland
Housing:	Two females per cage until GD 20 (except during mating), then one female per cage
Temperature:	22° ± 2°C
Humidity:	55% ± 10%
Air changes:	Not reported
Photoperiod:	12-hour dark/light cycle
Acclimation:	At least 3 weeks

B. PROCEDURES AND STUDY DESIGN

This study was designed to assess the potential of Ro 17-0099/000 to cause developmental toxicity in rats when administered daily by gavage on GDs 6-15, inclusive.

Mating

Females were mated in a ratio of 1 to 1 with resident males of the same strain (occasionally two females were mated with one male). The day on which a copulatory plug was observed was designated as gestation day (GD) 0.

Animal Assignment

Animal assignment and dose selection are presented in Table 1. Assignment was random but the report did not state how randomization was achieved.

TABLE 1. Animal Assignment[a]

Group	Dose Level (mg/kg/day)	Number of Animals Assigned
I	0	36
II	50	36
III	200	36
IV	800	36

Dose Selection Rationale

Doses were selected based on the results of two range-finding studies (Study Nos. 727R90 and 738R90). Results of these studies were not submitted but the following information was supplied: Animals received the test material at doses of 0, 15, 60, 200, or 800 mg/kg/day. Maternal and developmental toxicity were observed at 800 mg/kg/day as evidenced by decreased body weight gain and an increase in postimplantation loss, respectively.

Dosing

All doses were in a volume of 10 mL/kg of body weight based on the most recently recorded body weight data. Mixtures of the test material in the vehicle were prepared once at the beginning of the dosing period. Analyses for concentration and stability (14 days at 4°C) were conducted once and found to be acceptable.

C. OBSERVATIONS

Maternal Observations and Evaluations

The animals were checked once daily for mortality, moribundity, and clinical signs of toxicity. Body weights were recorded on GDs 0, 6 to 16, and 20 and on LDs 1, 4, 12, and 23. On GD 20, a minimum of 20 females (with litters) per group were sacrificed by carbon dioxide asphyxiation and litters were delivered by cesarean section. The remaining females were allowed to deliver their litters.

Examination of the dams at sacrifice on GD 20 included the following:

- Gross pathology examination of kidney, lung, and liver
- Number of corpora lutea
- Number of implantation sites
- Number of resorptions (early and late) and of live and dead fetuses

Examination of the dams at weaning included the following:

- Gross pathology examination of internal organs

Examination of the dams at weaning included the following:

- Number of implantation sites
- Gross pathology examination of uterine tissues

Uteri from apparently nonpregnant females were stained with ammonium sulfide to detect early embryonic loss.

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Fetal Evaluations

Examination of the fetuses on GD 20 included the following:

- External examination of all fetuses
- Visceral examination of one half of the fetuses using the technique described by Barrow and Taylor (1969)
- Skeletal examination of the remaining half of the fetuses using the technique described by Dawson (1926)

Pup Evaluations

Examination of the pups during lactation included the following:

- Individual weight at birth and on lactation days (LDs) 1, 4, 12, and 23
- Individual sex at birth and on day 23
- External examination of all pups. Pups with external abnormalities were processed for either visceral or skeletal examinations. All others were discarded after the external examination.

Statistical Analysis

The following statistical methods were employed:

- Categorical variables--Chi-square test and Fisher's exact test
- Normally distributed data--ANOVA and Dunnett's test
- Non-normally distributed data--Kruskall-Wallis test and Mann-Whitney-Wilcoxon test

Compliance

Signed and dated Good Laboratory Practice and Quality Assurance statements were provided.

RESULTS

A. MATERNAL TOXICITY

Mortality

No compound-related mortality was observed.

Clinical Observations

Compound-related clinical signs were observed at 800 mg/kg/day. They included piloerection (nine rats), poor general condition (three rats), encrustation around the nose (two rats), and encrustation around the mouth (three rats).

Body Weight

A summary of body weight gain data is presented in Table 2. Compound-related effects were observed at 800 mg/kg/day. At this dose level, mean body weight gain decreased significantly during the dosing period to 48% of control. Mean body weight at 800 mg/kg/day were also decreased significantly ($p \leq 0.01$; data not shown) on GD 16 to 93% of control. Gravid uterine weights were not recorded for the dams selected for cesarean sectioning, and consequently, mean corrected weight gain was not determined. Body weights and weight gains were not affected during lactation.

TABLE 2. Body Weight Gain (g ± S.D.)[a]

Dose Group (mg/kg/day)	Pre-Dosing Period (GDs 0-6)	Dosing Period (GDs 6-16)	Post Dosing Period (GDs 16-20)
0	21.8 ± 4.4	35.1 ± 6.3	44.4 ± 10.9
50	22.7 ± 4.6	37.2 ± 6.9	45.1 ± 8.8
200	23.3 ± 4.4	33.7 ± 7.4	48.0 ± 9.4
800	22.4 ± 4.4	17.0 ± 23.6***	43.7 ± 19.0

[a]Data extracted from Study No. B-154'980, Table 5
***Significantly different from control, $P<0.001$

Food Consumption

Food consumption was not determined.

Gross Pathology Observations

No compound-related gross findings were observed.

Cesarean Section and Natural Delivery Observations

Cesarean section and natural delivery data are summarized in Table 4. No compound-related effects were observed.

B. DEVELOPMENTAL TOXICITY

Summaries of incidental fetal external, visceral, and skeletal major abnormalities are presented in Tables 5a, b, and c, respectively. No compound-related major abnormalities, variations, or retardations were observed. Among pups allowed to survive to weaning, one pup from the control group had an incompletely opened eyelid and one pup from the 800-mg/kg/day group had a malformed head (data not shown).

TABLE 4. Cesarean Section and Natural Delivery Observations[a]

Observation	Doses (mg/kg/day) 0	50	200	800
# Animals assigned	36	36	36	36
# Animals pregnant	36	34	34	34
Pregnancy rate (%)[b]	100	94	94	94
Maternal wastage				
# Died	1	0	0	0
# Died/pregnant	0	0	1	0
# Non pregnant	0	2	2	2
# Aborted	0	0	0	0
# Premature delivery	0	0	0	0
Cesarean Section Observations				
# Dams/litters evaluated	21	20	20	22
Total corpora lutea	310	288	288	331
Corpora lutea/dam	14.8 ± 2.2[c]	14.4 ± 2.1	14.4 ± 2.0	15.0 ± 2.5
Total implantations	273	252	271	291
Implantations/dam	13.0 ± 3.5	12.6 ± 1.9	13.6 ± 1.7	13.2 ± 2.0
Total live fetuses	241	231	249	239
Live fetuses/dam	11.5 ± 3.6	11.6 ± 2.8	12.4 ± 2.3	10.9 ± 4.0
Total resorptions	32	21	22	50
Early	32	18	22	34
Late	0	3	0	16
Resorptions/dam	1.5 ± 1.9	1.0 ± 1.3	1.1 ± 1.2	2.3 ± 3.7
Total dead fetuses	0	0	0	2
Dead fetuses/dam	0	0	0	0.1 ± 0.4
Mean fetal weight (g)	3.3 ± 0.2	3.4 ± 0.2	3.4 ± 0.2	3.4 ± 0.5
Preimplantation loss (%)	12	13	6	12
Postimplantation loss (%)	12	8	8	18
Sex ratio (% male)	54	55	50	51
Natural Delivery Observations				
# Dams/litters evaluated	15	13	14	11
Duration of gestation	21.9	21.8	21.9	22.2
Total implantation sites	184	181	184	152
Implantations/litter	12.3 ± 3.9	13.9 ± 1.6	13.1 ± 3.9	13.8 ± 1.5
Total resorptions	18	12	14	23
Percentage resorptions	10	7	8	15
Live pups/litter				
LD 1	10.9 ± 4.0	13.0 ± 1.6	12.1 ± 3.7	11.3 ± 2.0
LD 4	10.4 ± 3.9	12.7 ± 1.9	11.6 ± 4.0	11.0 ± 2.0
LD 12	10.1 ± 3.8	12.0 ± 2.0	11.0 ± 3.6	10.9 ± 2.1
LD 23	10.1 ± 3.8	11.9 ± 2.1	11.0 ± 3.6	10.9 ± 2.1
Pup weight/litter				
LD 1	5.5 ± 0.6	5.3 ± 0.3	5.3 ± 0.5	5.6 ± 0.4
LD 4	7.4 ± 1.2	7.0 ± 0.8	7.2 ± 0.8	7.9 ± 0.8
LD 12	17.7 ± 2.7	16.6 ± 1.6	17.9 ± 2.0	20.0 ± 2.1
LD 23	37.8 ± 6.6	35.4 ± 3.2	37.1 ± 5.4	42.3 ± 5.3
Sex ratio (% male)	48	54	55	48

[a]Data extracted from Study No. B-154'980, Tables 1, 9, and 17
[b]Calculated by reviewers, not statistically analyzed
[c]Mean ± S.D.

TABLE 5a. External Examination[a]

Observations[b]	Dose Groups (mg/kg/day) 0	50	200	800
# Pups examined	241	231	249	241
# Litters examined	21	20	20	20
Ectopy of intestines	0	0	2 (2)[c]	1
Anasarca	0	2 (1)	0	0
Diastasis recti abdominis	0	0	0	1
Ears displaced	0	1	0	0
Exencephaly	0	0	0	1
Lower jaw shortened	1	0	0	0
Exophthalmia	0	0	0	1
Total with any external abnormalities	1	2 (1)	2 (2)	3 (1)

[a]Data extracted from Study No. B-154'980, Tables 11 and 12
[b]Some observations may be grouped together
[c]Number of litters

TABLE 5b. Visceral Examination[a]

Observations[b]	Dose Groups (mg/kg/day) 0	50	200	800
# Pups examined	107	110	118	115
# Litters examined	20	20	20	20
Protruding tongue	0	0	0	1
Shortened upper jaw	0	0	0	1
Dilation, lateral ventricles	7 (5)[c]	8 (6)	1*	1*
Dilation, 3rd ventricle	2 (2)	1	0	2 (2)
Complete interruption of aortic arch	1	0	0	0
Situs inversus	0	0	0	1
Diaphragmatic hernia	0	2 (1)	0	1
Displaced liver	0	1	0	0
Missing kidney	0	0	2 (1)	0
Hydronephrosis	1	0	1	0
Enlarged adrenals	0	0	1	0
Hydroureter	2 (2)	0	1	1
Missing ureter	0	0	2 (1)	0
Absence of uterine horn	0	0	1	0
Total with any visceral abnormalities	10 (8)	10 (7)	4 (3)	6 (4)

[a]Data extracted from Study No. B-154'980, Tables 13 and 14
[b]Some observations may be grouped together
[c]Number of litters
*Significantly different from control, $p<0.05$

TABLE 5c. Skeletal Examination[a]

Observations[b]	Dose Groups (mg/kg/day) 0	50	200	800
# Pups examined	118	121	131	126
# Litters examined	20	20	20	20
Generalized reduction of bone size	0	0	1	0
Cleft palate	0	0	0	1
Exocipital fused with cervical vertebral arches	0	0	0	1
Lumbar vertebral arch fused with center, 6X	0	0	1	0
Sternal element misshaped	1	1	0	0
Sternal elements fused	0	0	1	0
Rib misshaped	2 (2)[c]	5 (4)	1	0
Rib missing	0	0	0	1
Fibula misshaped	0	1	0	0
Hyperflexion of hindlimbs	0	1	1	0
Total with any skeletal abnormalities	3 (3)	7 (6)	4 (4)	1

[a]Data extracted from Study No. B-154'980, Tables 15 and 16
[b]Some observations may be grouped together
[c]Number of litters

DISCUSSION

A. MATERNAL TOXICITY

Compound-related maternal toxicity was observed at 800 mg/kg/day as evidenced by an increased incidence of clinical signs (piloerection, poor general condition, and encrustation of mouth and nose) and decreased body weight and weight gain during the dosing period.

B. DEVELOPMENTAL TOXICITY

No developmental (deaths/resorptions, altered growth, or anomalies) compound-related effects were observed. Among the groups selected for cesarean sectioning, the number of late resorptions was slightly increased above control at 800 mg/kg/day. This increase was not considered to be an effect of the test compound since it was statistically nonsignificant and other developmental endpoints were not affected at this dose level.

C. STUDY DEFICIENCIES

The age of the animals was not reported. Gravid uterine weights were not recorded. A protocol was not submitted. These deficiencies did not impact upon the outcome of the study.

D. CORE CLASSIFICATION: Core Guideline Data.

Maternal NOEL = 200 mg/kg/day
Maternal LOEL = 800 mg/kg/day based on an increased incidence of clinical signs and decreased body weight/weight gain

Developmental Toxicity NOEL = 800 mg/kg/day
Developmental Toxicity LOEL = not determined